ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of the Anti-Bacterial and Anti-Fungal Activity of *Callicarpa arborea* Leaves.

Umachandur*, Battu Ganga Rao, Kalyani ALT, and Ramadevi Devarakonda.

Andhra University College of Pharmaceutical Sciences, Andhra University, Vishakapatnam,-530003, Andhra Pradesh, India.

ABSTRACT

Callicarpa species Family: Verbenaceae has been a source of antimicrobials. The purpose of the present study is to evaluate the antibacterial and antifungal activities of the hydro-alcoholic (70% alcohol in water) extract of its leaves. The zone of inhibition by cup plate method was determined at concentrations (100, 200, and 300 mg/ml) and compared to that of the standards-Amikacin (0.01mg/ml) for antibacterial and Fluconazole (0.01mg/ml) for antifungal. The test organisms used were Gram Positive bacteria -*Staphylococcus aureus, Bacillus subtilis,* Gram Negative bacteria- *Proteus vulgaris, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa,* fungal strains: *Candida albicans, Aspergillus niger, Aspergillus oryzae, Penicillium chrysogenum.*Fluconazole exhibited a zone of inhibition of 51-52 mm and the extracts: 29-34 mm against fungal strains. Amikacin exhibited a zone of inhibition of 42 mm in gram negative organisms and 45 mm in gram positive organisms, whereas the extracts exhibited a zone of inhibition of 24-28 mm. From the above results, it can be concluded that the hydro alcoholic extract of the leaves of *Callicarpa arborea* exhibited significant antifungal activity and, antibacterial activity against both gram positive and gram negative bacteria, in a dose dependant manner as compared to that of the standard.

Keywords: *Callicarpa arborea*, leaves, anti-bacterial, antifungal, zone of inhibition.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The development of microbial resistance to the currently available anti microbials has made it necessary to search for new ones. Plants are a source of potential antibacterial and antifungals which may provide a solution to the problem [1]. Beautyberry (*Callicarpa*) is a genus of shrubs and small trees in the family Verbenaceae, consisting of 40-150 species. They are native to east and Southeast Asia (where the majority of the species occur), Australia, North America and Central America [2]. The genus *Callicarpa* has been a source of anti-microbials and Extracts from about 14 species in this genus have been evaluated for biological activity, including antibacterial, antifungal activities [3-5]. *Callicarpa arborea* bark and leaf have been used in traditional and folklore medicine in the treatment of wounds and insect stings and skin diseases [6,7]. The purpose of the study is therefore to evaluate the anti-fungal and anti-bacterial activity of the leaf extract (70% ethanol in water) of *Callicarpa arborea* .

MATERIALS AND METHODS:

Collection and authentication of the plant material

The leaves of *Callicarpa arborea* were collected from Araku valley Paderu region in Visakhapatnam district of Andhra Pradesh .The plant material was identified by Prof. Dr.Venkaiah, Dept. of Botany, Andhra University, Visakhapatnam. A voucher specimen of the authenticated sample was deposited in the A.U.B.D.H.) bearing No. 21113.

Preparation of the extract

The hydro alcoholic (70% ethanol in water) extract was prepared by subjecting the shade dried and powdered leaf to soxhlet extraction for 72 hours. The extract was further evaporated to dryness on a water bath.

Test Organisms

The microorganisms used for the experimental work were procured from MTTC (Microbial Type Culture Collection) I MTECH (Institute of Microbial Technology) Chandigarh, Punjab, India.

The test organisms used were

- Gram Positive bacteria -*Staphylococcus aureus, Bacillus subtilis*
- Gram Negative bacteria- *Proteus vulgaris, Escherichia coli, Klebsiella pneumoniae, Pseudomonas Aeruginosa*
- Fungal strains: *Candida albicans, Aspergillus niger, Aspergillus oryzae, Penicillium chrysogenum*

Culture Media

Nutrient Agar and Sabaraud's Dextrose agar was obtained from Hi –Media.

Standards

Mikacin (Amikacin 100 mg, 1 Unit in vial); Aristo Pharma, and Diflucan (fluconazole 2mg/ml) vial: Pfizer; were obtained from the local medical store.

Extracts

The extracts were dissolved in ethyl acetate and diluted to obtain the required concentrations.

ISSN: 0975-8585

METHOD

The Anti-bacterial and anti-fungal activity was determined by the standard agar cup plate method [8,9].

The antibacterial activity was determined as follows

One ml of the standardized bacterial stock suspension (10^8 - 10^9) C.F.U/ ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45˚C. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petridishes .The agars were left to set and in each of these plates 4 cups (6 mm in diameter) were cut using a sterile cork borer and agar discs were removed. Alternate cups were filled with 0.05 ml samples of each of the extracts and the standard using a micropipettette, and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37˚C for 24 hours. Simultaneously positive controls involving the addition of the respective solvents instead of the extracts were carried out separately. After incubation the diameters of the resultant growth inhibition zones were measured in millimetres.

The Anti-fungal Activity was determined in the same manner as the anti-bacterial activity using Sabarauds dextrose agar medium with an incubation period of 48 hours.

RESULTS

The results of the study can be tabulated as follows:

Table 1: Zone of Inhibition against gram positive and gram negative bacteria

Sample	Conc mg/ml	Zones of inhibition (Diameter in mm)					
		Gram Positive		Gram Negative			
		Staphylococcus aureus	Bacillus subtilis	Proteus vulgaris	Escherichia coli	Klebsiella pneumoniae	Pseudomonas aeruginosa
CAL	100	25	24	21	22	26	24
CAL	200	27	26	24	25	24	26
CAL	300	28	28	25	28	26	27
Standard Amikacin	0.01	45	45	42	42	42	42

CAL-70 % hydro alcoholic extract of *Callicarpa arborea*

Table 2: Zone of Inhibition against Fungal Strains

Sample	Concentration mg/ml	Zones of inhibition (Diameter in mm)			
		Candida albicans	*Aspergillus niger*	*Aspergillus oryzae*	*Penicillium chrysogenum*
CAL	100	29	31	30	29
CAL	200	31	33	32	31
CAL	300	34	34	34	33
Standard Fluconazole	0.01	52	51	51	51
Ethyl acetate (Vehicle)	-	-	-	-	-

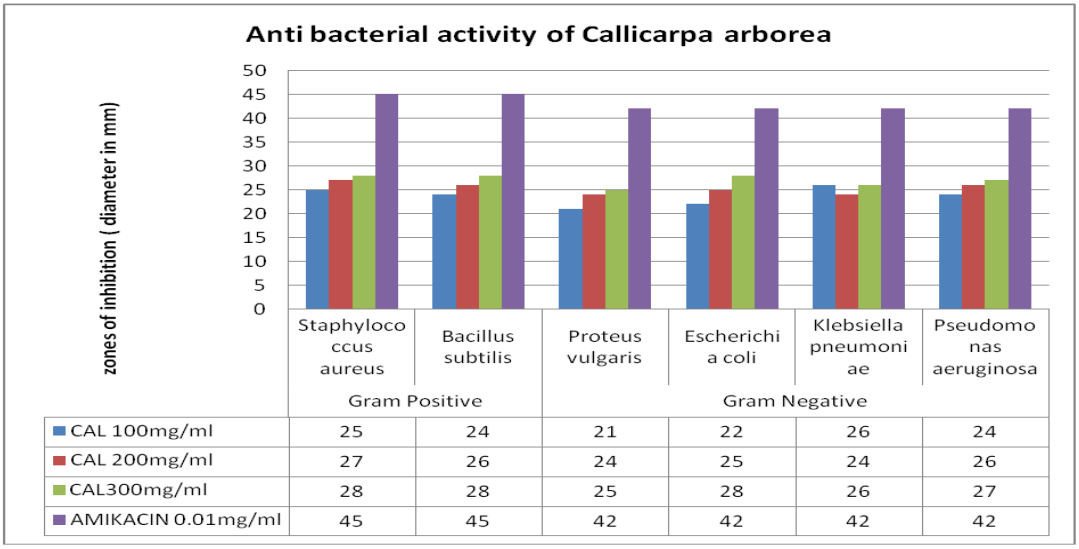
CAL-70 % hydro alcoholic extract of *Callicarpa arborea*

To summarize, the standard fluconazole exhibited a zone of inhibition of 51-52 mm and the extracts: 29-34 mm against fungal strains .The standard Amikacin exhibited a zone of inhibition of 42 mm in case of gram negative organisms and 45 mm in case of gram positive organisms, whereas the extracts exhibited a zone of inhibition of 24-28 mm.

ISSN: 0975-8585

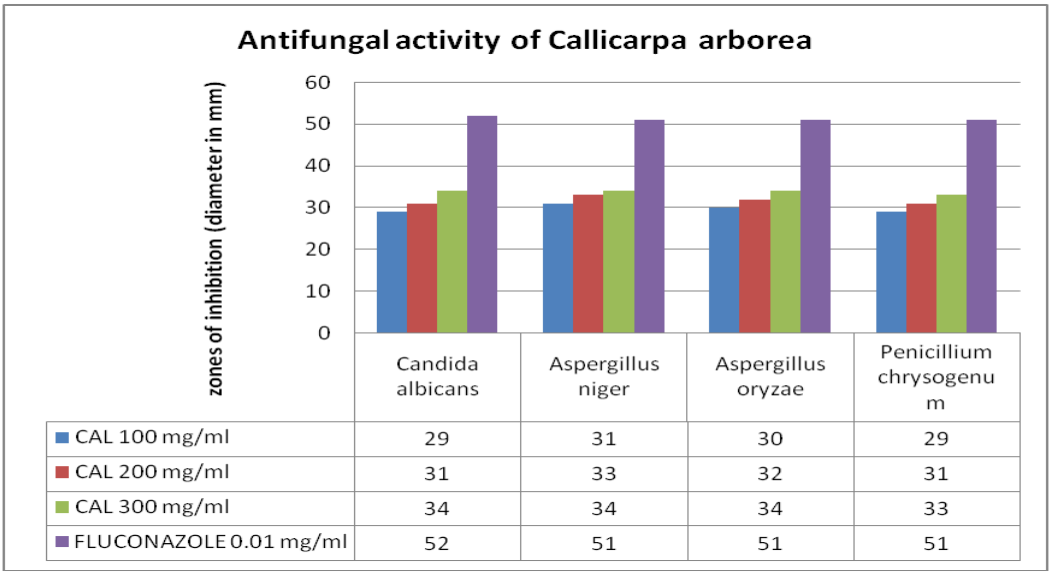
The results can be represented graphically as follows:

Graph 1: Anti- bacterial activity of *Callicarpa arborea*



	Staphylococcus aureus	Bacillus subtilis	Proteus vulgaris	Escherichia coli	Klebsiella pneumoniae	Pseudomonas aeruginosa
CAL 100mg/ml	25	24	21	22	26	24
CAL 200mg/ml	27	26	24	25	24	26
CAL300mg/ml	28	28	25	28	26	27
AMIKACIN 0.01mg/ml	45	45	42	42	42	42

Graph 2: Anti- Fungal activity of *Callicarpa arborea*



	Candida albicans	Aspergillus niger	Aspergillus oryzae	Penicillium chrysogenum
CAL 100 mg/ml	29	31	30	29
CAL 200 mg/ml	31	33	32	31
CAL 300 mg/ml	34	34	34	33
FLUCONAZOLE 0.01 mg/ml	52	51	51	51

DISCUSSION

From the above results, it can be concluded that, the hydro alcoholic extract of the leaves of *Callicarpa arborea* exhibited moderately significant antifungal and, antibacterial activity against both gram positive and gram negative bacteria, in a dose dependant manner, as compared to that of the standard.

Thus, Callicarpa arborea leaves provide a source of anti-microbial phytocompounds which can be investigated further. However, the determination of the MIC (minimum inhibitory concentration) and testing the activity on highly resistant strains of bacteria would be needed to further enhance the value of the work.

ACKNOWLEDGEMENTS

I gratefully acknowledge Mr.R.Rajendran, CEO, Green Chem, Bangalore, for his help in the preparation of the extracts. I also thank Dr.Srinivas Prof Venkaiah, Dept. of Botany, Andhra University, Dr.Prayaga Murthy, Mr. Srinivas Duvvada, and Mr.Praneeth Dasari for their help in procuring the plant material. I also take this

opportunity to thank. Mrs. A.L.T.Kalyani, Research Scholar, Dept. of Biotechnology, Andhra University College of Pharmaceutical Sciences, without whose help this work would not have been possible.

REFERENCES

[1] Pesaramalli Karteek et al. Int Res J Pharm 2012;3(8):230-232.
[2] www. wikepedia.org
[3] William P Jones and A Douglaskinghorn. 2008 ; 4(1):15–32.
[4] http://www.webmedcentral.com/article_view/3651.
[5] Virendra Yadav, Soma Jayalakshmi, Rajeev K Singla, Arjun Patra. Indo Global Journal of Pharmaceutical Sciences 2012;(2): 103-107.
[6] RC Srivastava. Indian J Trad Knowl 2009;8(2):146-153.
[7] M Venkaiah, P Prayaga Murthy, SB Padal. The Useful Plants of Andhra Pradesh,. First Edition, September 2010 India Publisher- K Jayalakshmi. Page 53.
[8] Greenwood D, Slack RCB and Peutherer JF. In; Medical microbiology 14 thEdn, ELBS, London, 1992, 1.
[9] Bauer AW, et al. Am J Clin Path1966;45:493.